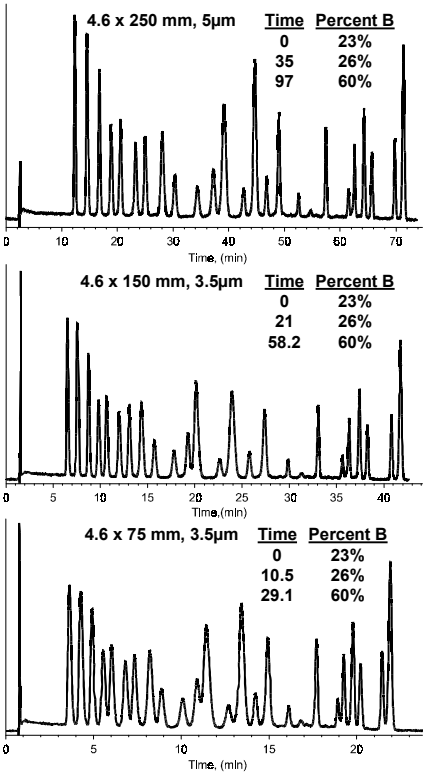
# High-Efficiency and High-Speed Separation of Natural Anthocyanins

## Application

### Food Analysis

Robert Ricker

Anthocyanins are natural pigments responsible for the brilliant red and blue (and purple) colors found in many fruits and flowers. The colors of blueberries are the result of many different anthocyanins being present in the fruit. Qualitative and quantitative analysis of anthocyanins can be used to distinguish between different cultivars of blueberry plants and determine their quality. Therefore the chromatographic separation of anthocyanins is of increasing importance to the agricultural and wine industries. Recent interest in medicinal use of anthocyanins, as antioxidants/anticancer agents, has also stimulated interest in their chromatographic separation.




**Conditions:**
**ZORBAX SB-C18 (4.6 x 250 mm, 5 µm; 4.6 x 150 mm, 3.5 µm; 4.6 x 75 mm; 3.5 µm)**
**(Agilent P/N: 880975-902, 863953-902, 866953-902, respectively)**
**Mobile Phase: A:3% Phosphoric acid B: 100% MeOH, Gradient: (see individual chromatogram)**
**Inj.: 20 µL, 1 mL/min, 30°C, Detect. UV(525 nm), Sample preparation: (see back)**

***Highlights***

- *Traditionally, a low-pH mobile phase (containing formic acid) in these types of separations has caused degradation of the column and change in the separation (Goiffon, J.-P., Brun, M., and Bourrier, M.-J., J. Chromatogr. 537 101-121, 1991). ZORBAX StableBond SB-C18 columns provide the chromatographer with long-term stability for reverse-phase separations requiring very low pH.*
- *In these experiments, phosphoric acid has replaced the traditional formic acid in the mobile phase (Gao, L. and Mazza, G., J. Liq. Chromatogr., 18(2) 245-259, 1995), resulting in a superior separation of >25 anthocyanins on the ZORBAX SB-C18 column.*
- *As a result of reproducible products and smaller chromatographic particles (3.5µm) of narow size-distribution, the analyst can systematically change column configuration to save both time and solvent.*

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***Method of Preparing Blueberry Extracts****

Begin my mixing: 10 g. Blueberries
10mL Solvent (70:28:2, MeOH:H2O, Formic acid)
Blend for 10 minutes on ice.
Filter through glass wool in a 10 mL syringe.
Allow filtrate to sit for 1 hour.
Filter through a 0.2 µm filter.
Inject 50 µL immediately for HPLC analysis.

***Method of Extraction and blueberry extracts were kindly provided by Drs. Willy Kalt and Jane McDonald, Agriculture and Agri-Food Canada, Kentville, Nova Scotia, Canada***

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